PCT/US2004/011024

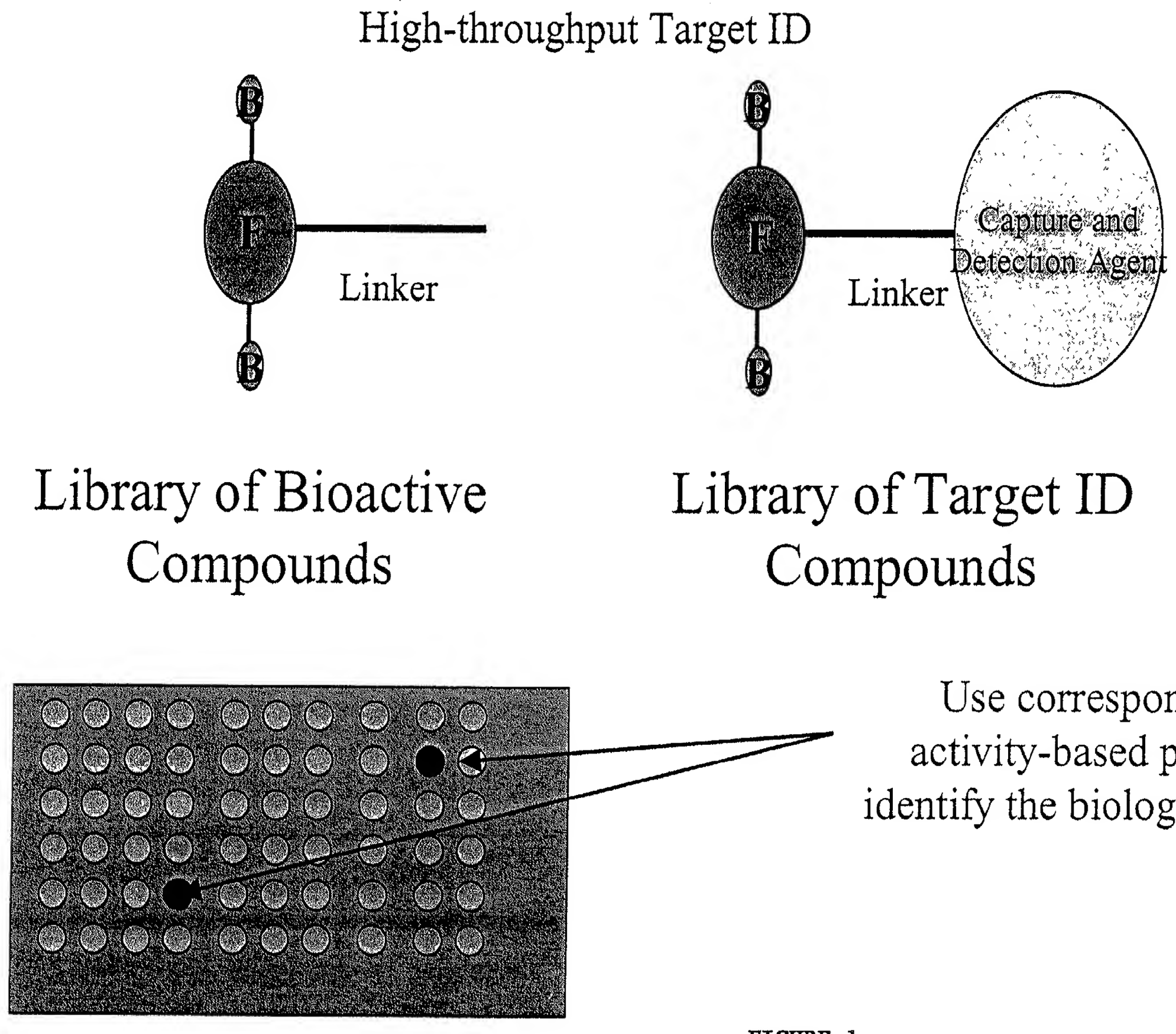


FIGURE 1

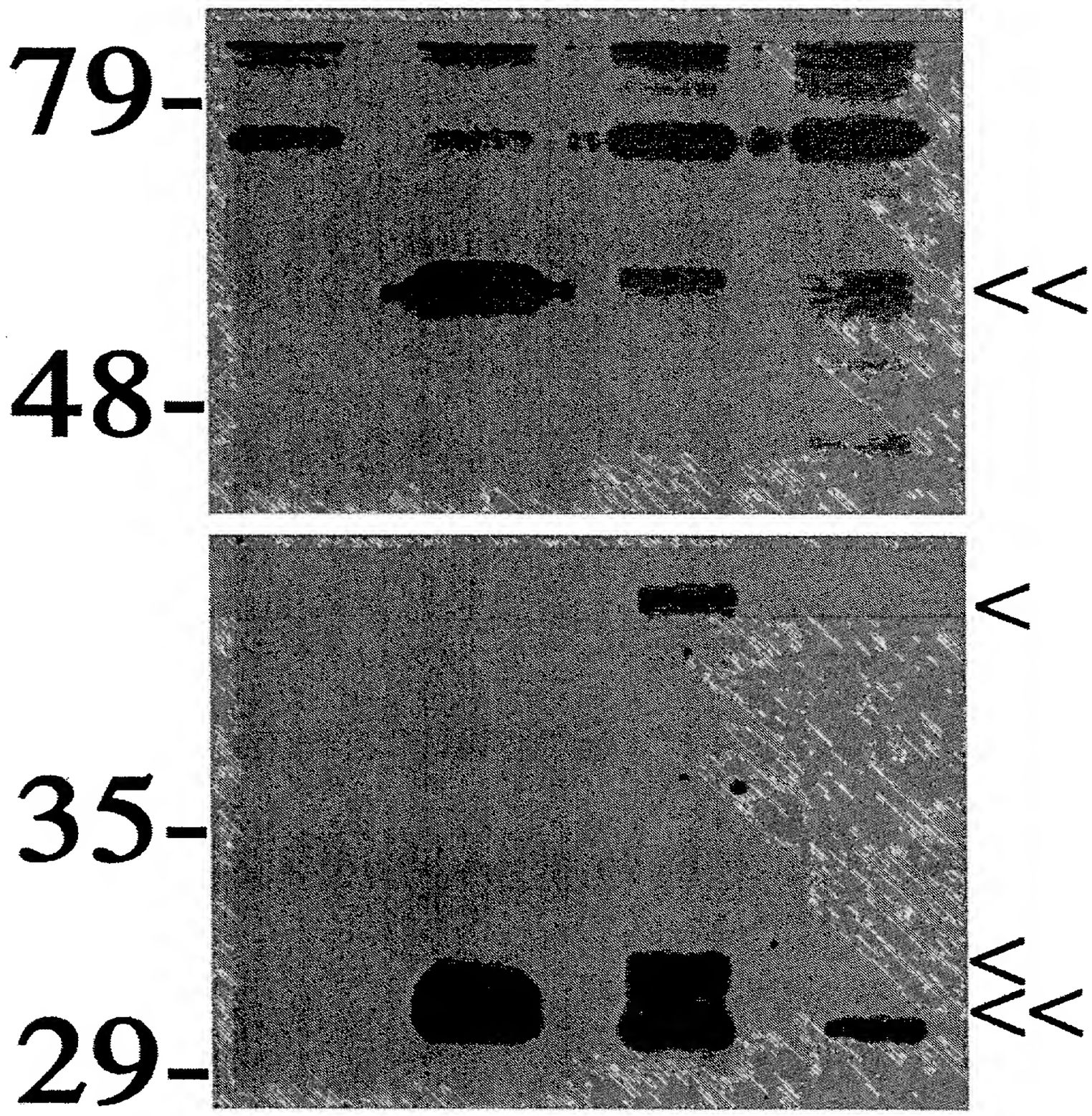
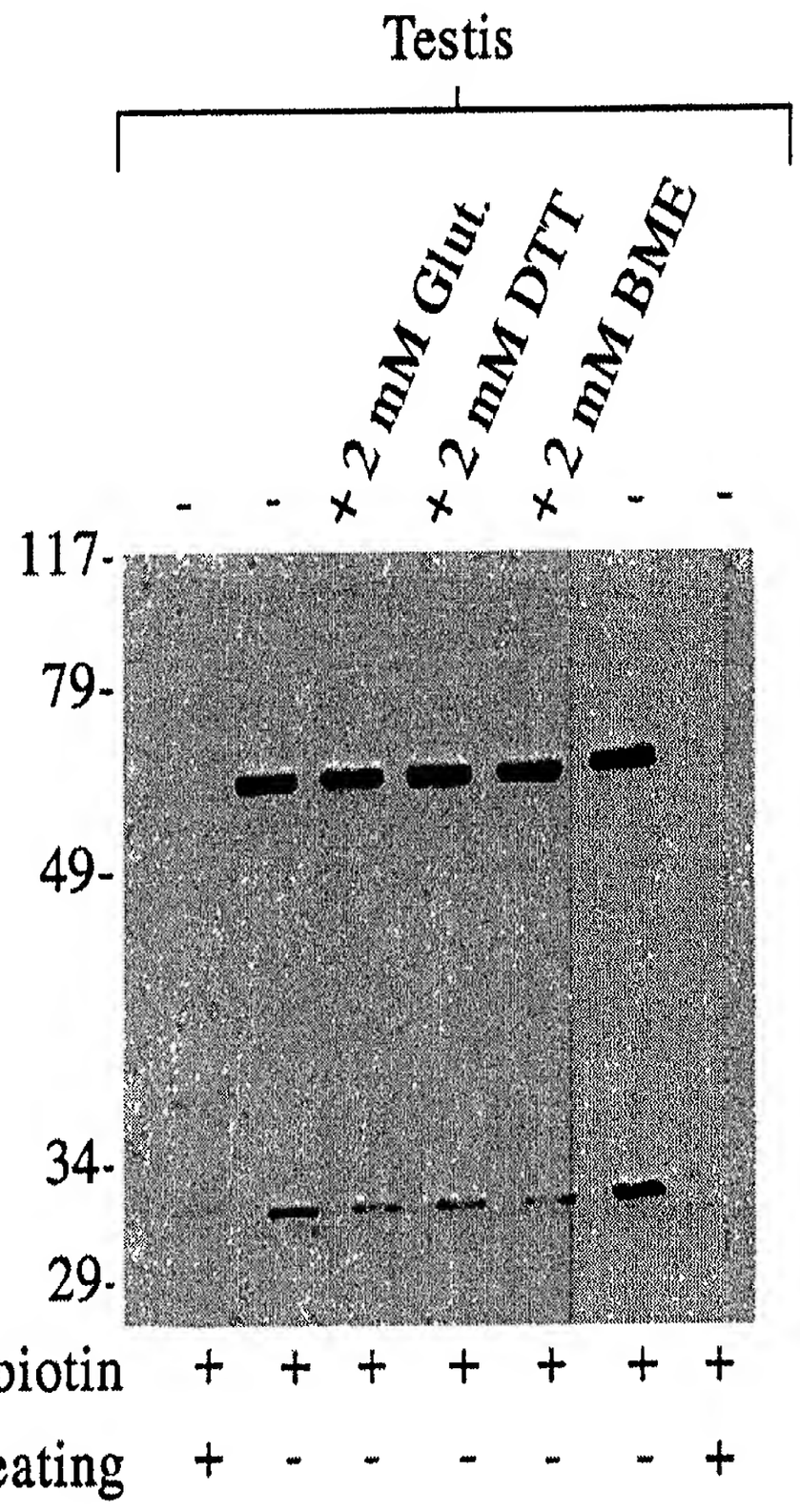


FIGURE 2

PLEASE INSERT



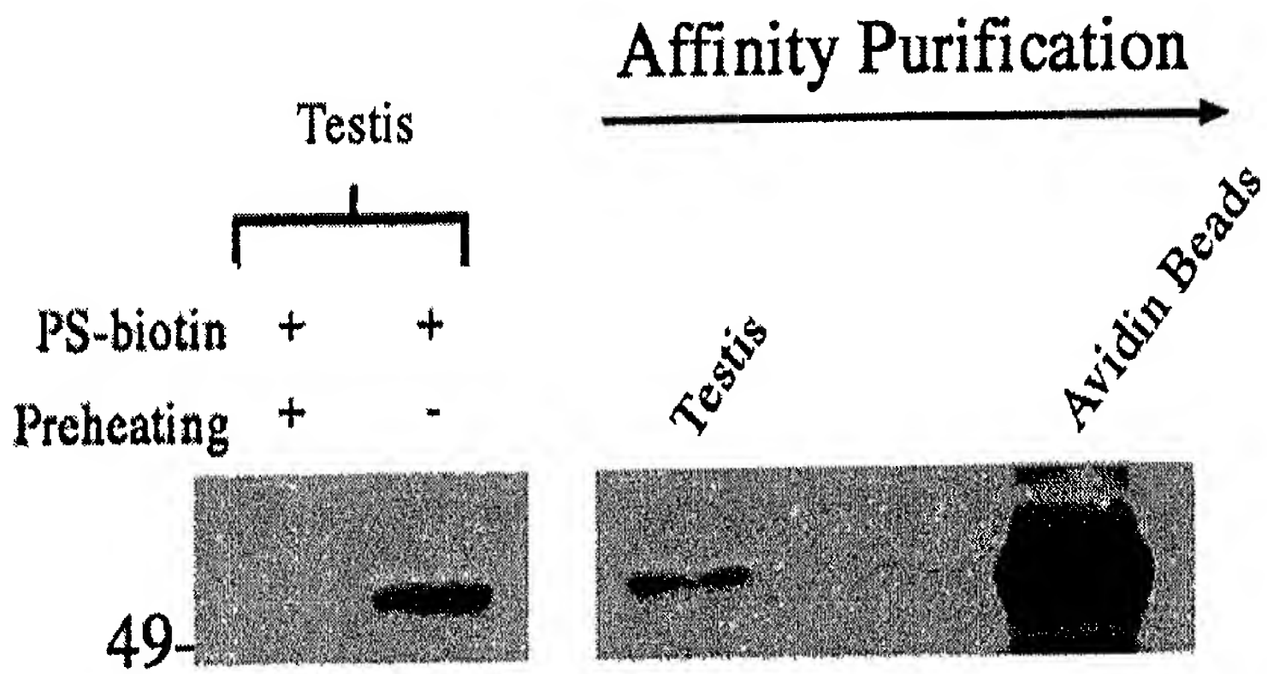
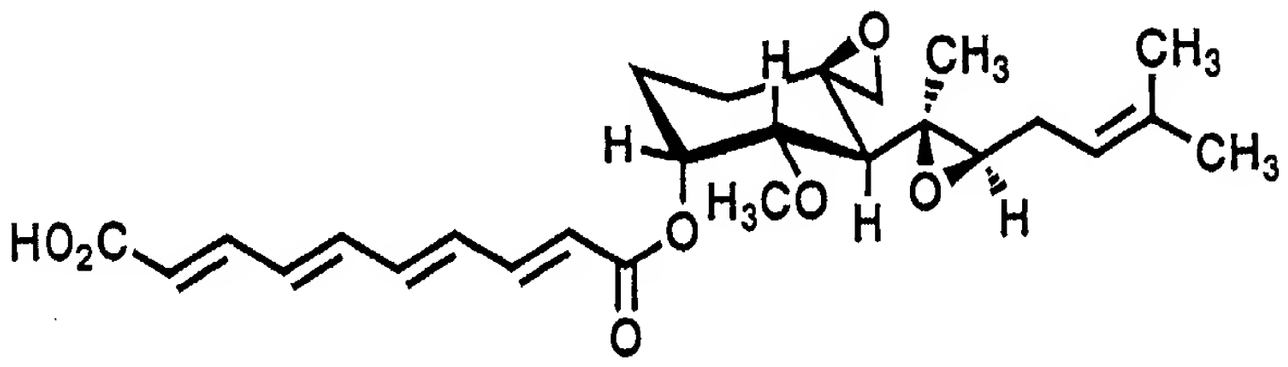
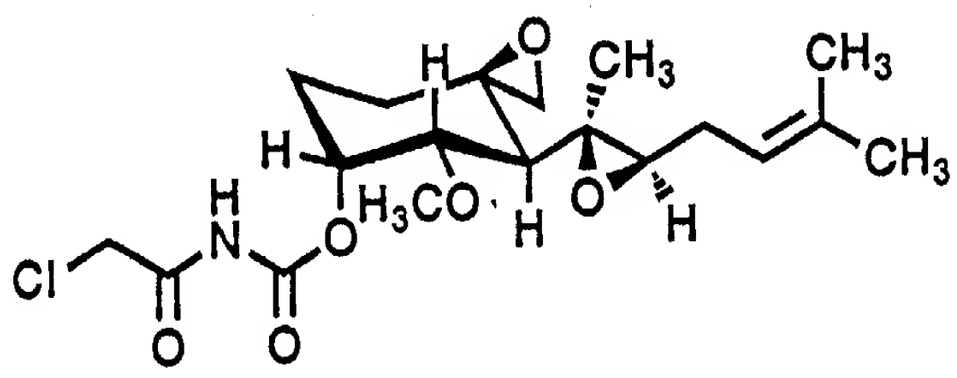


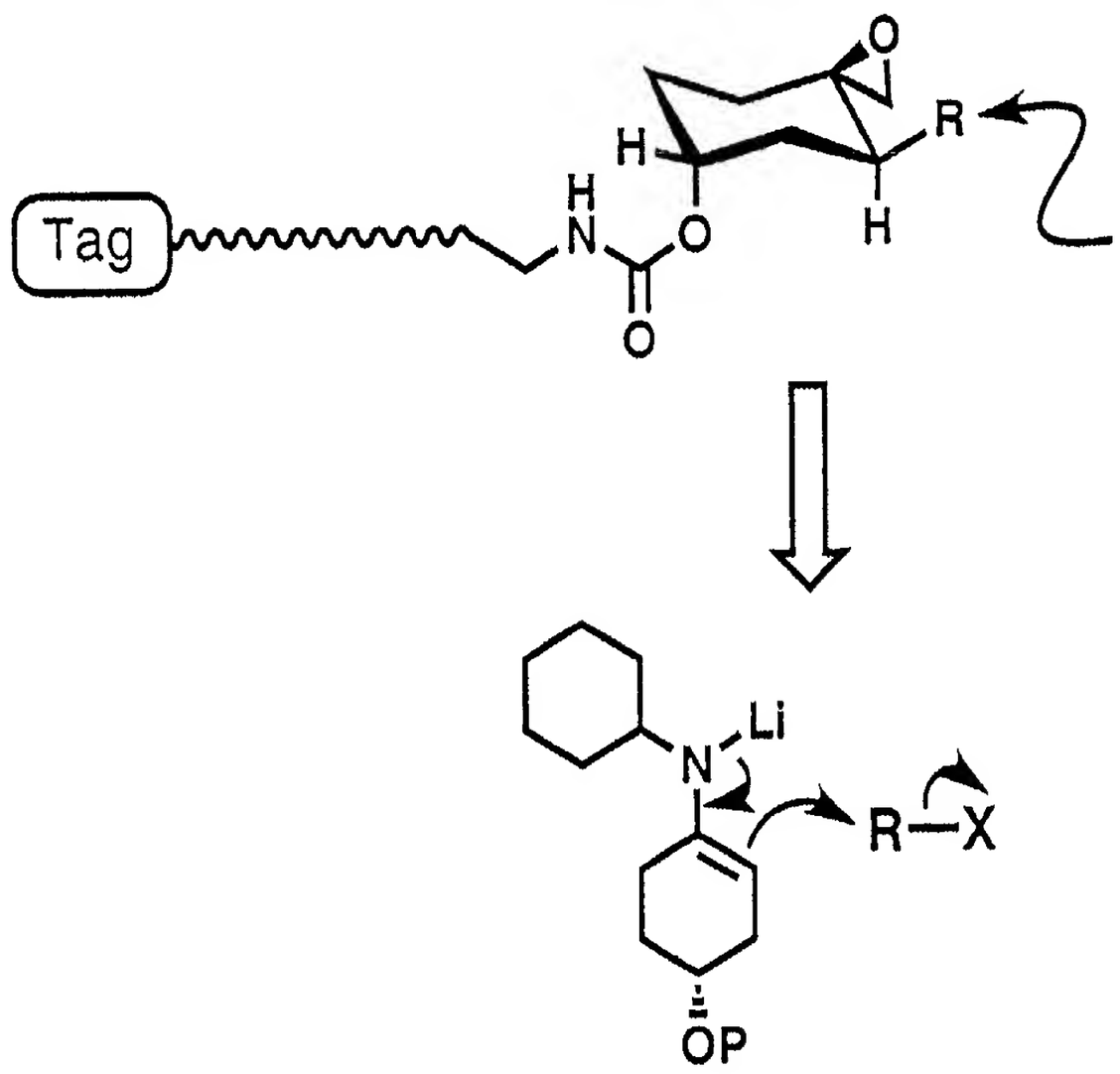
FIGURE 3



Fumagillin



TNP-470



X = halide
R = various aliphatic, alicyclic, alkyl aryl, or alkyl heteroaryl groups
P = unspecified protecting group

FIGURE 4A

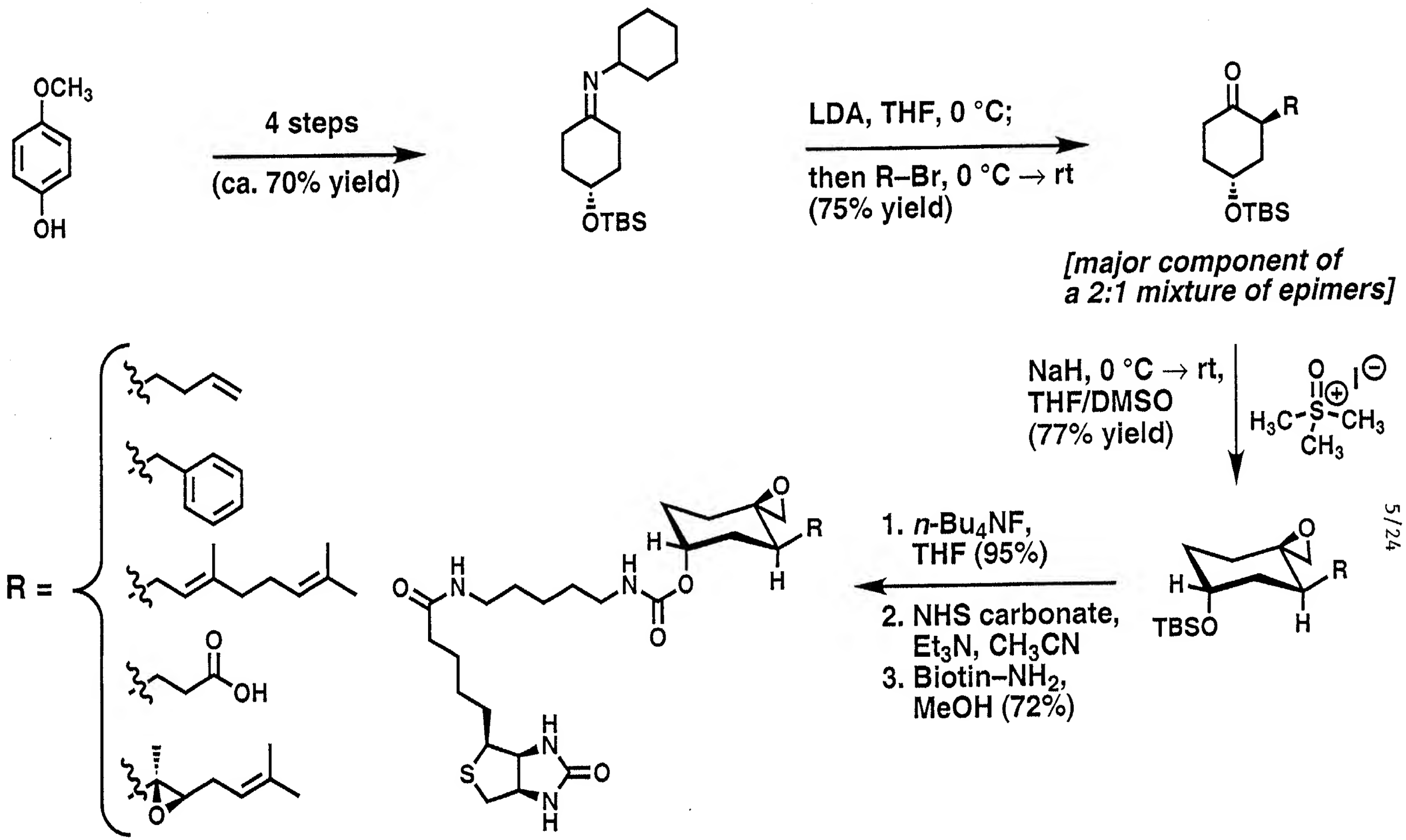


FIGURE 4B

5/24

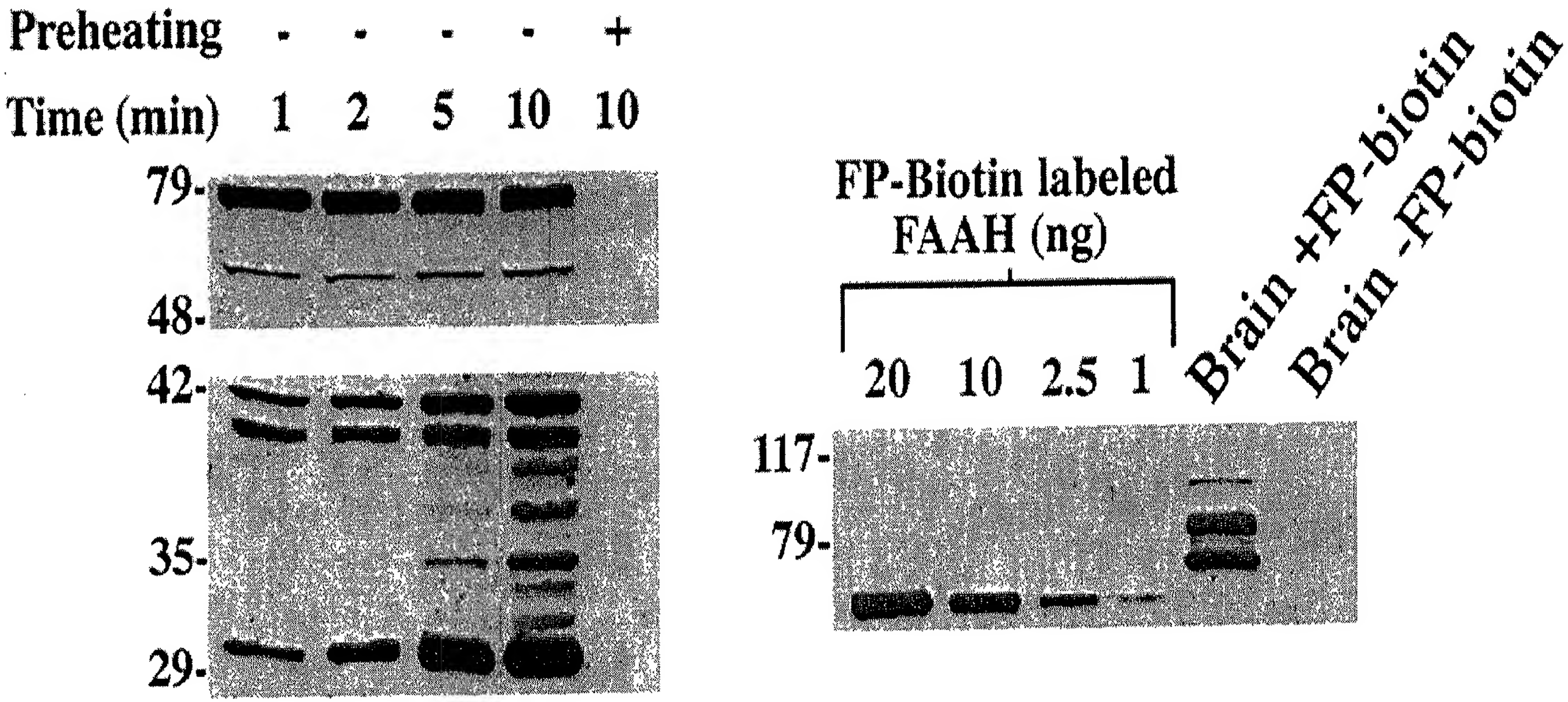
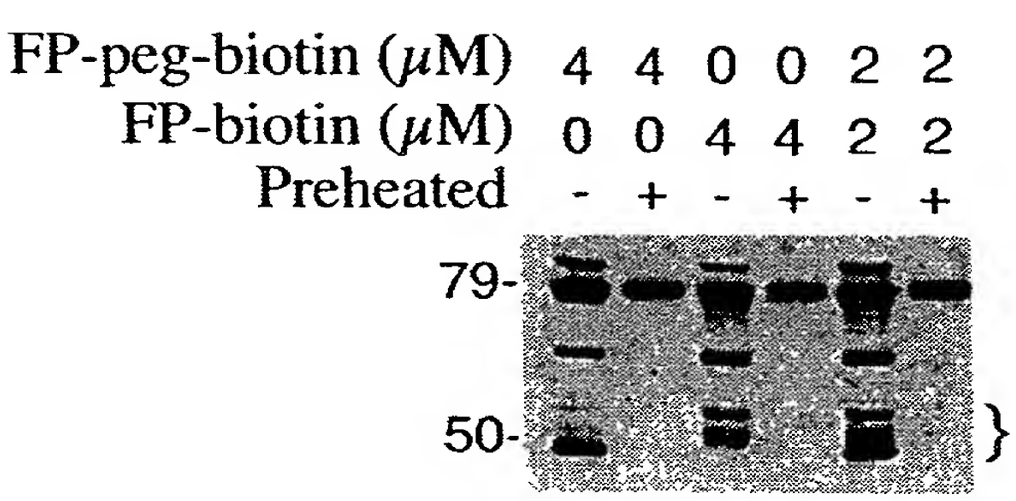

Preheating - - - - +
Time (min) 1 2 5 10 10
79-
48-
42-
35-
29-
FP-Biotin labeled FAAH (ng)
20 10 2.5 1
Brain +FP-biotin
Brain -FP-biotin
117-
79-

FIGURE 5


FP-peg-biotin (μM) 4 4 0 0 2 2
FP-biotin (μM) 0 0 4 4 2 2
Preheated - + - + - +
79-
50-
}

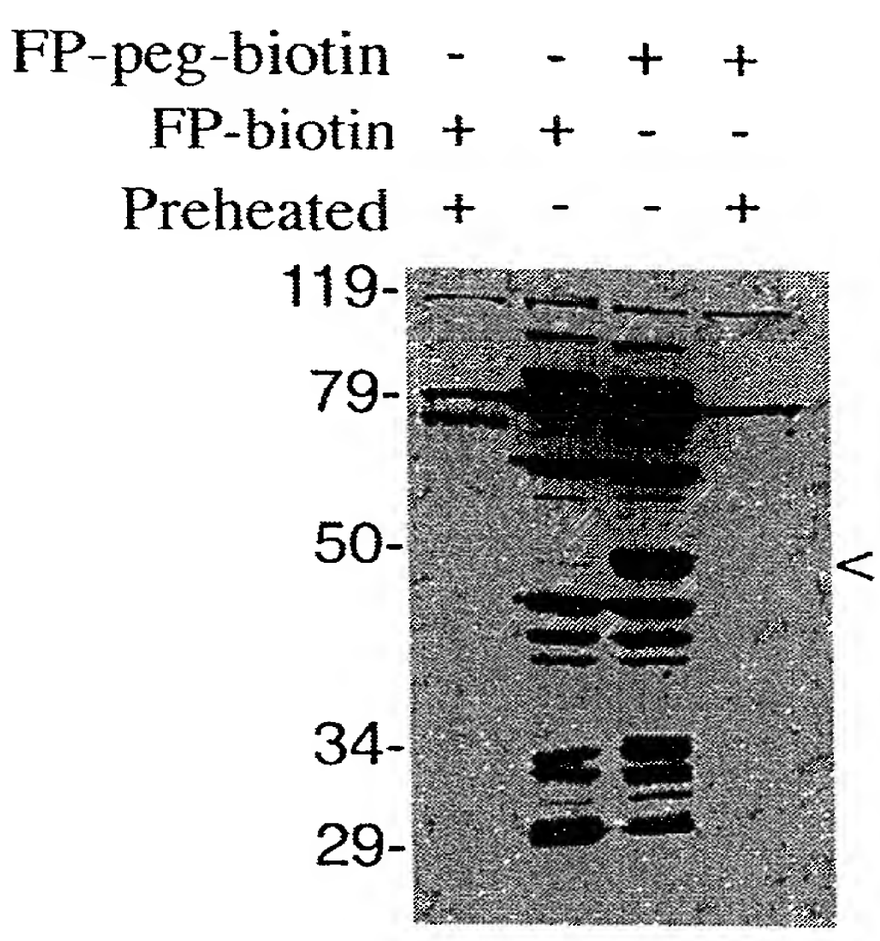

FP-peg-biotin - - + +
FP-biotin + + - -
Preheated + - - +
119-
79-
50-
34-
29-
<

FIGURE 6

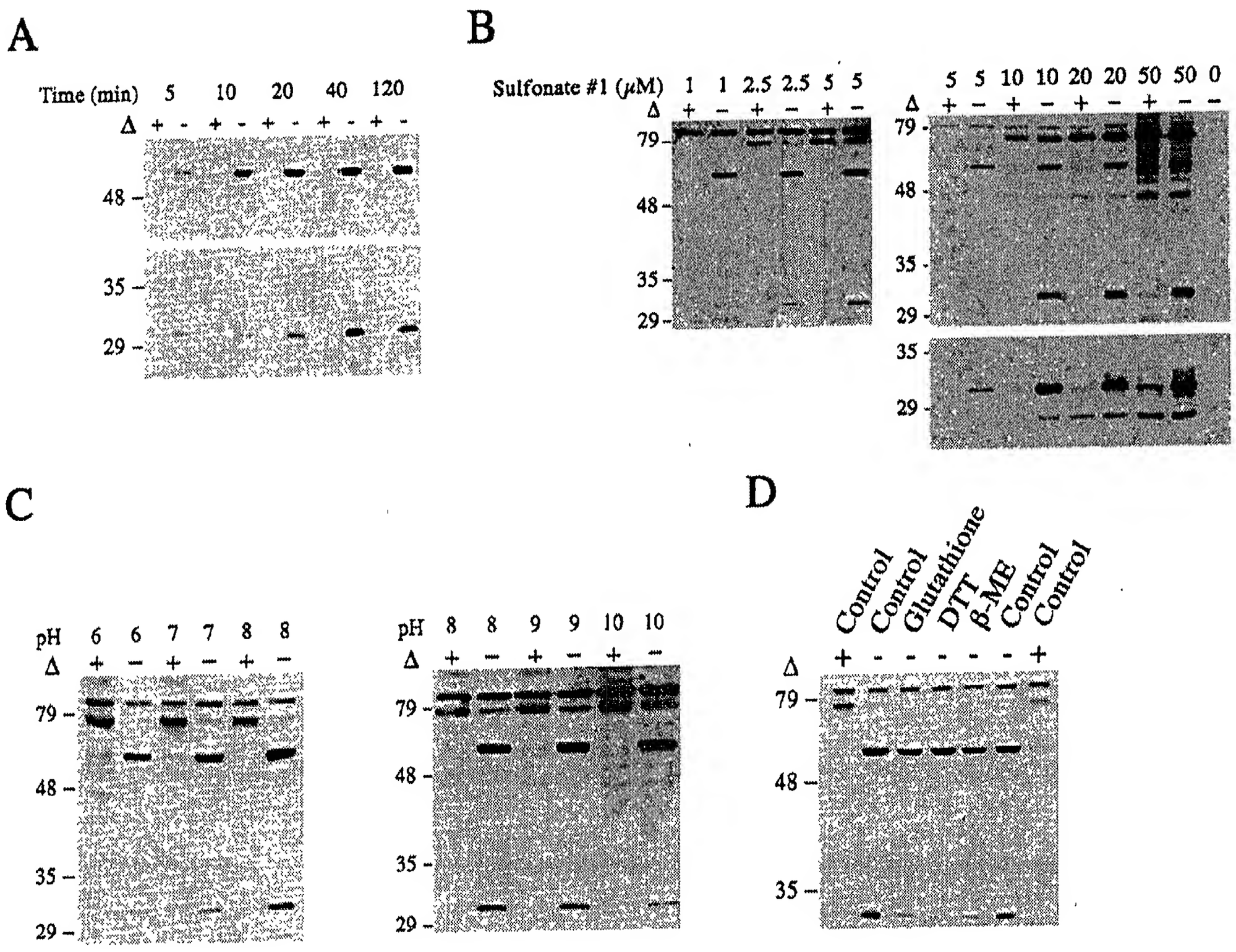

A
Time (min) 5 10 20 40 120
Δ + - + - + - + - + -
48
35
29
B
Sulfonate #1 (μM) 1 1 2.5 2.5 5 5
Δ + - + - + -
79
48
35
29
5 5 10 10 20 20 50 50 0
Δ + - + - + - + - -
79
48
35
29
35
29
C
pH 6 6 7 7 8 8
Δ + - + - + -
79
48
35
29
pH 8 8 9 9 10 10
Δ + - + - + -
79
48
35
29
D
Control
Control
Glutathione
DTT
β-ME
Control
Control
Δ + - - - - - +
79
48
35

FIGURE 7

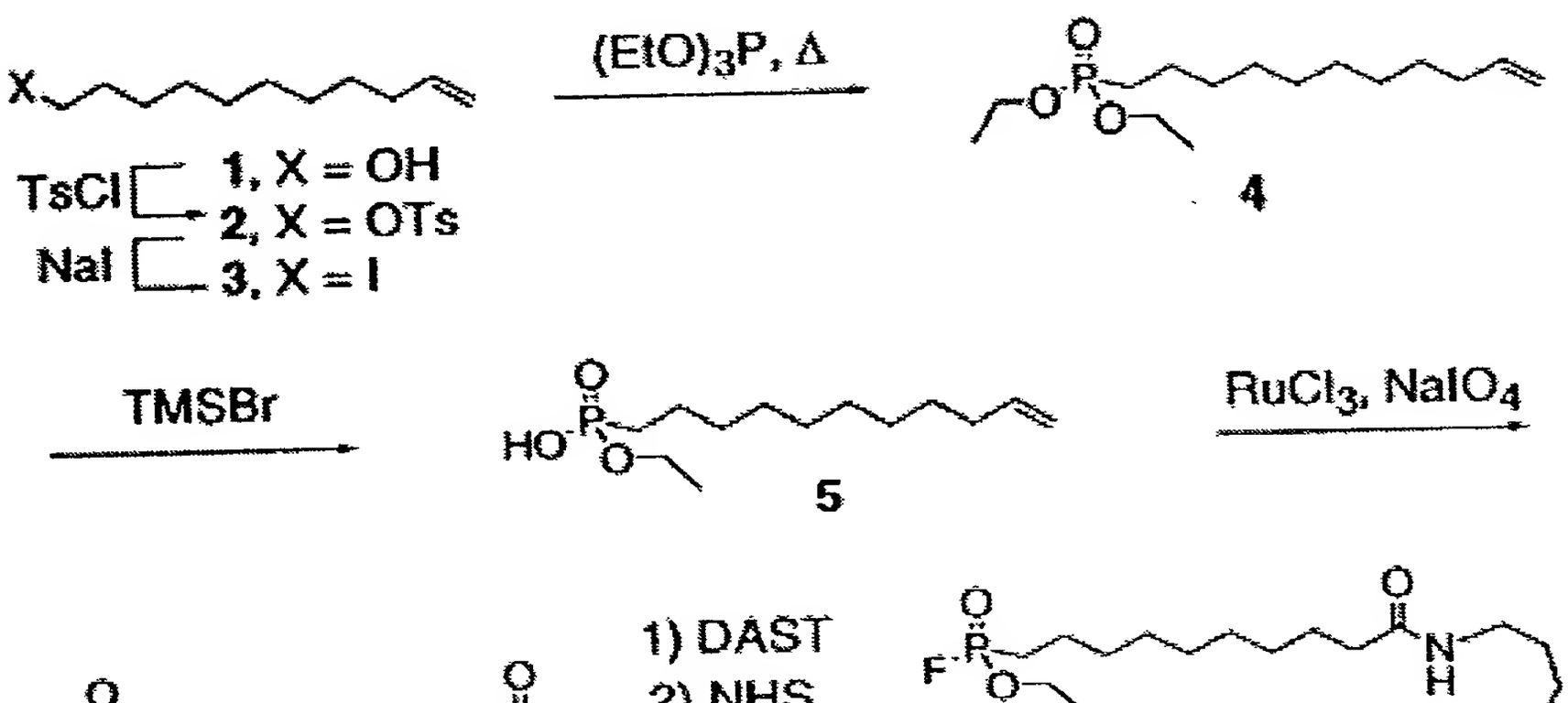

(EtO)3P, Δ
TsCl
NaI
1, X = OH
2, X = OTs
3, X = I
4
TMSBr
5
RuCl3, NaIO4
1) DAST
2) NHS
3) NH2-biotin
6
7, FP-biotin
8, FP-fluorescein

FIGURE 8

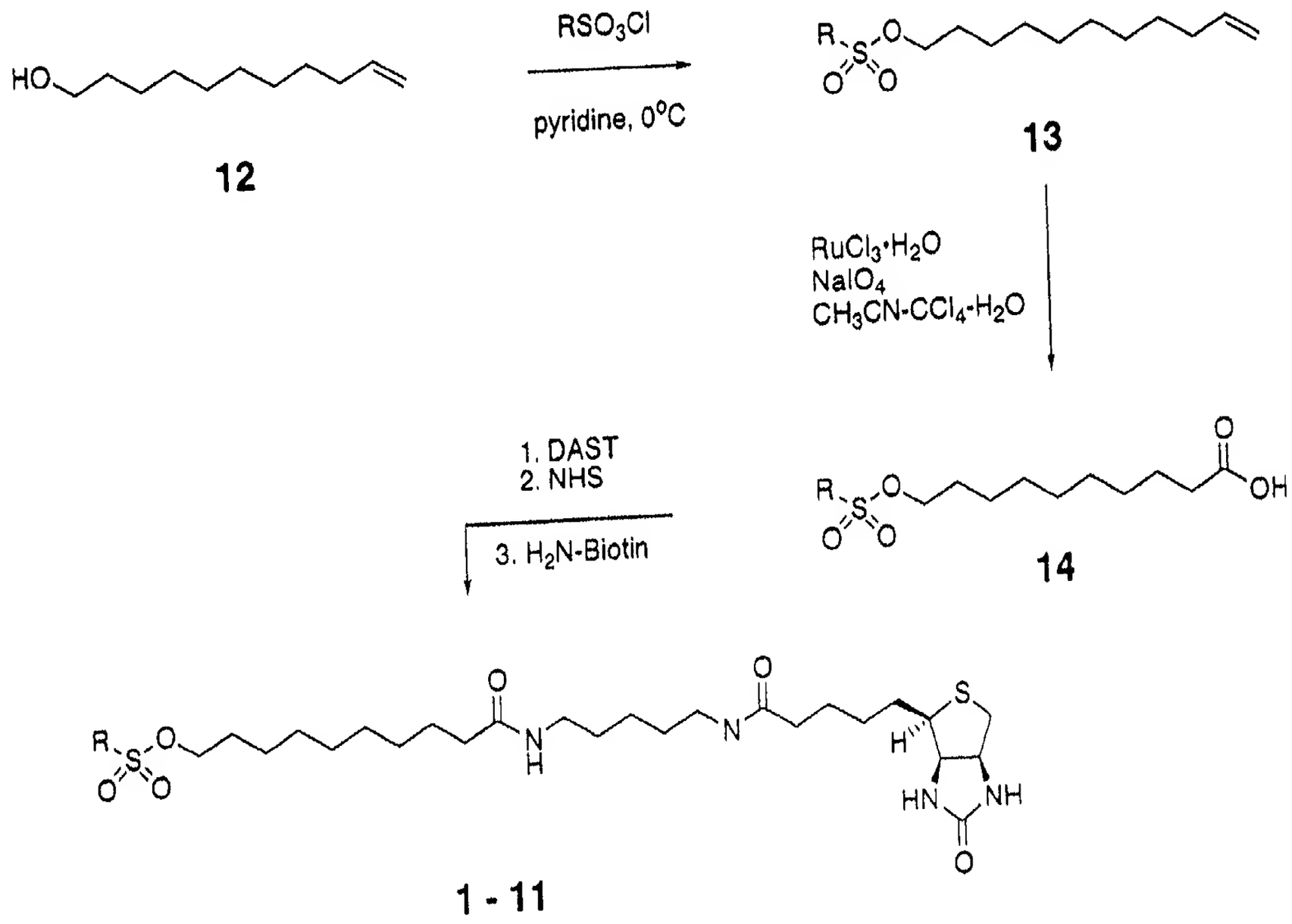
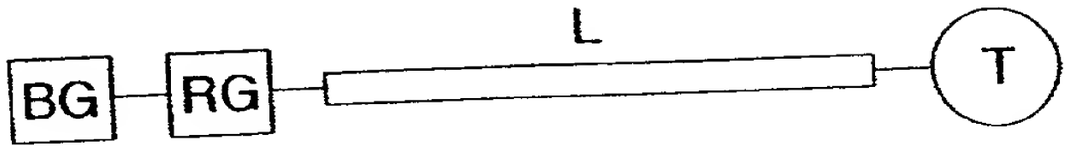

HO
12
RSO3Cl
pyridine, 0°C
R
S
O
13
RuCl3·H2O
NaIO4
CH3CN-CCl4-H2O
1. DAST
2. NHS
3. H2N-Biotin
OH
14
H
N
S
HN
NH
1 - 11

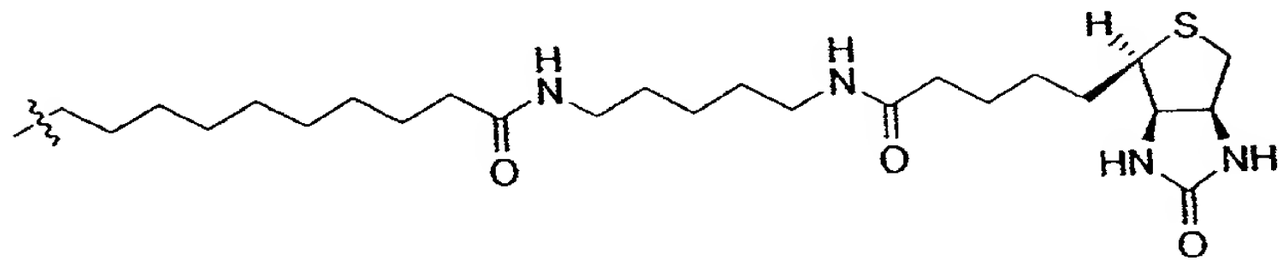
FIGURE 9

A.

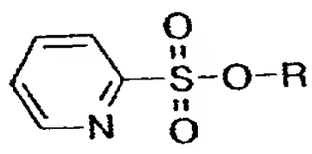


B.

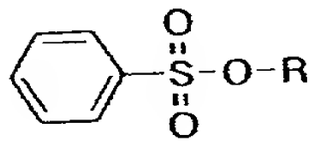
R =



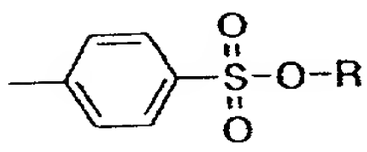
1



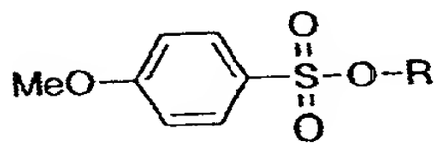
2



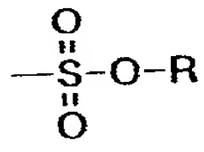
3



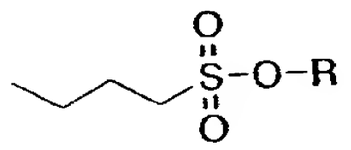
4



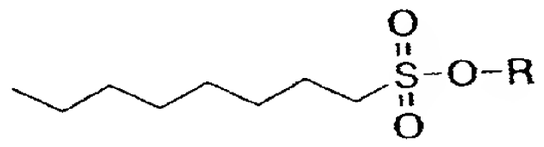
5



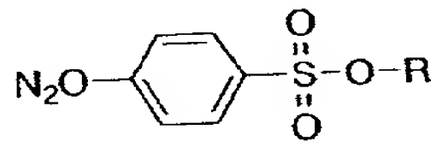
6



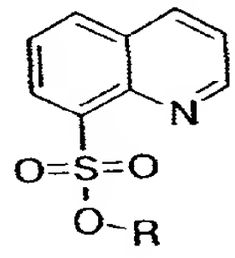
7



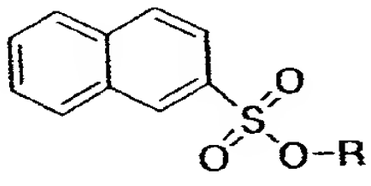
8



9



10



11

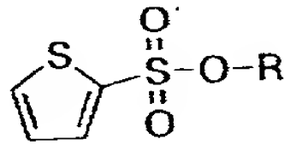


FIGURE 10

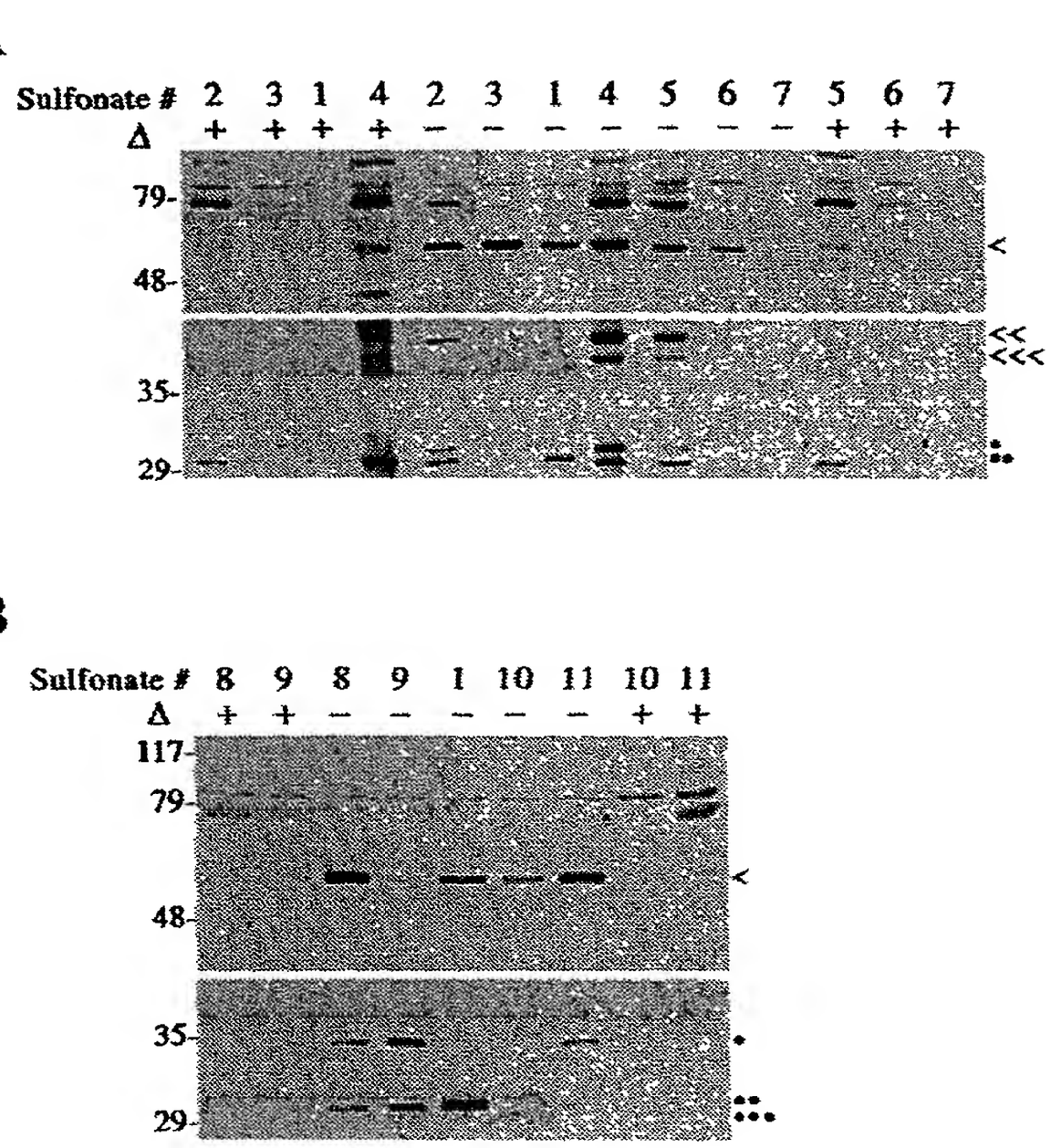

A
Sulfonate # 2 3 1 4 2 3 1 4 5 6 7 5 6 7
Δ + + + + − − − − − − − + + +
79-
48-
35-
29-
<
<<
<<<
•
••
B
Sulfonate # 8 9 8 9 1 10 11 10 11
Δ + + − − − − − + +
117-
79-
48-
35-
29-
<
•
••
•••

FIGURE 11

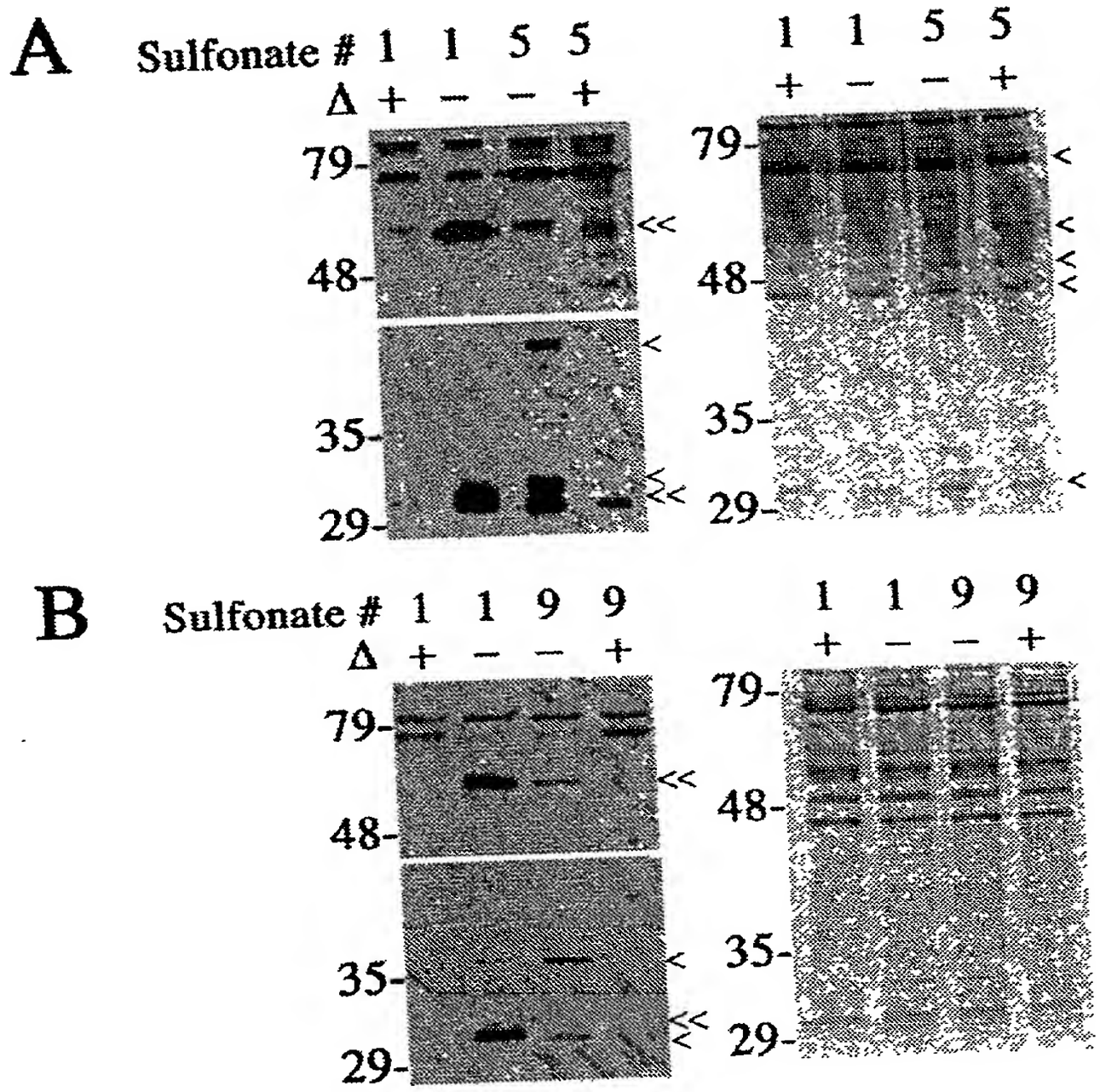
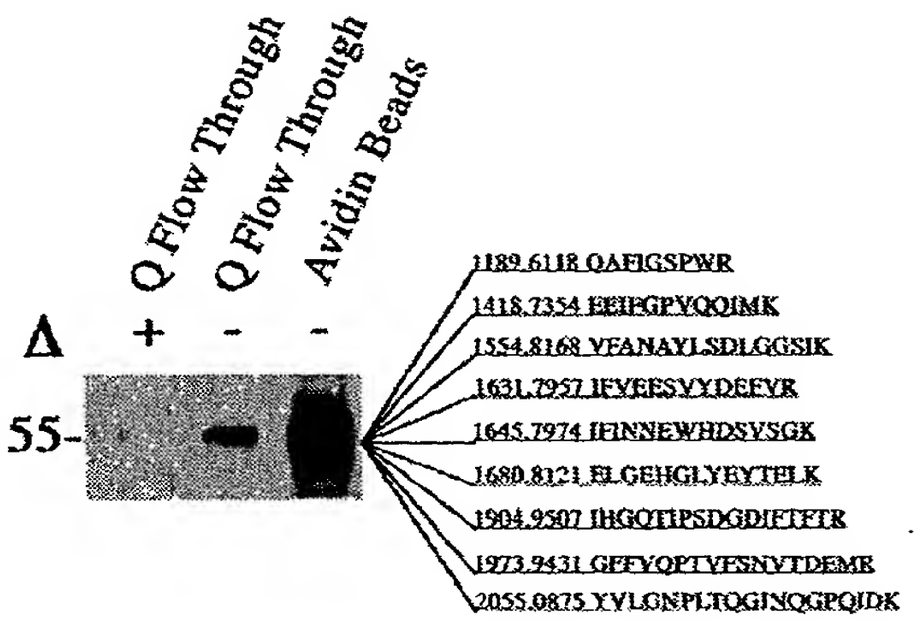
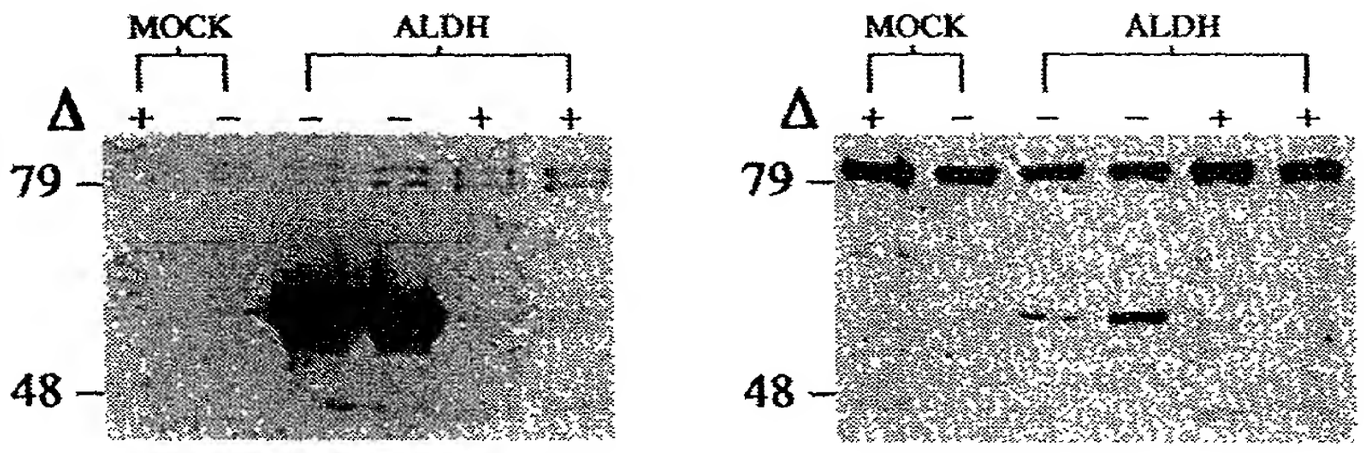

A
Sulfonate # 1 1 5 5
Δ + − − +
79-
48-
35-
29-
1 1 5 5
+ − − +
79-
48-
35-
29-
B
Sulfonate # 1 1 9 9
Δ + − − +
79-
48-
35-
29-
1 1 9 9
+ − − +
79-
48-
35-
29-

FIGURE 12

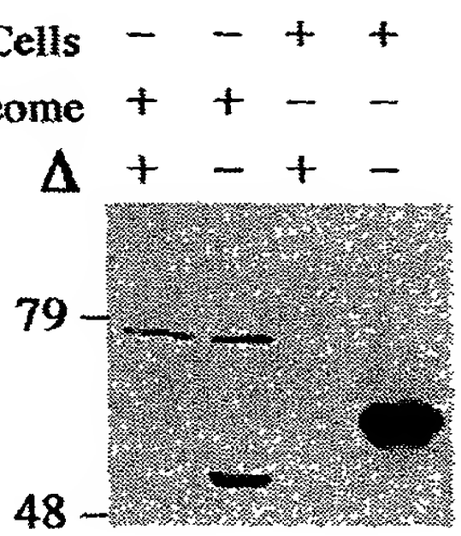
A



B



C



D

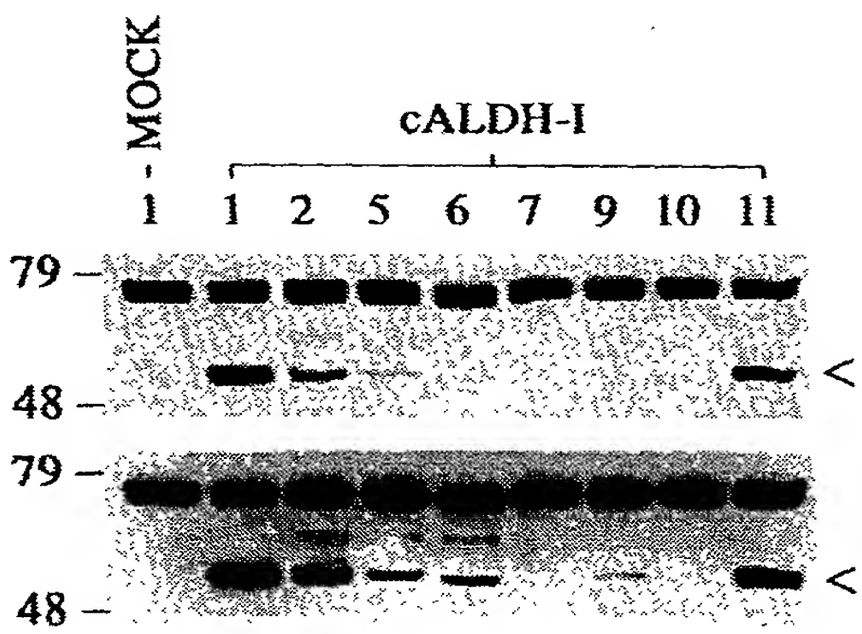
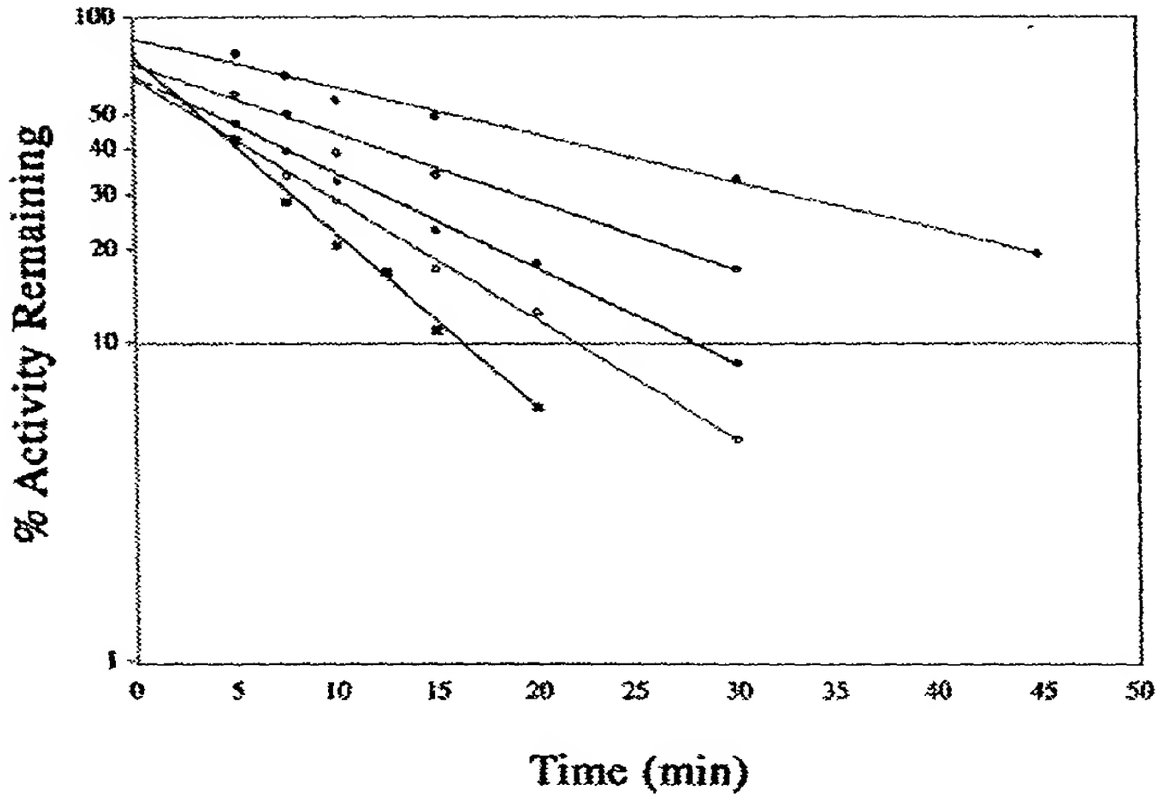


FIGURE 13

A



B

Competitor #	-	-	15	17	16	15	17	16
[Competitor (μM)]	0	0	5	5	5	50	50	50
Δ	+	−	−	−	−	−	−	−

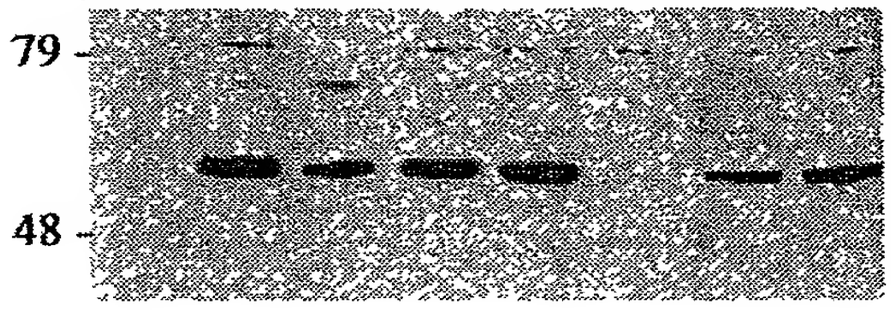


FIGURE 14

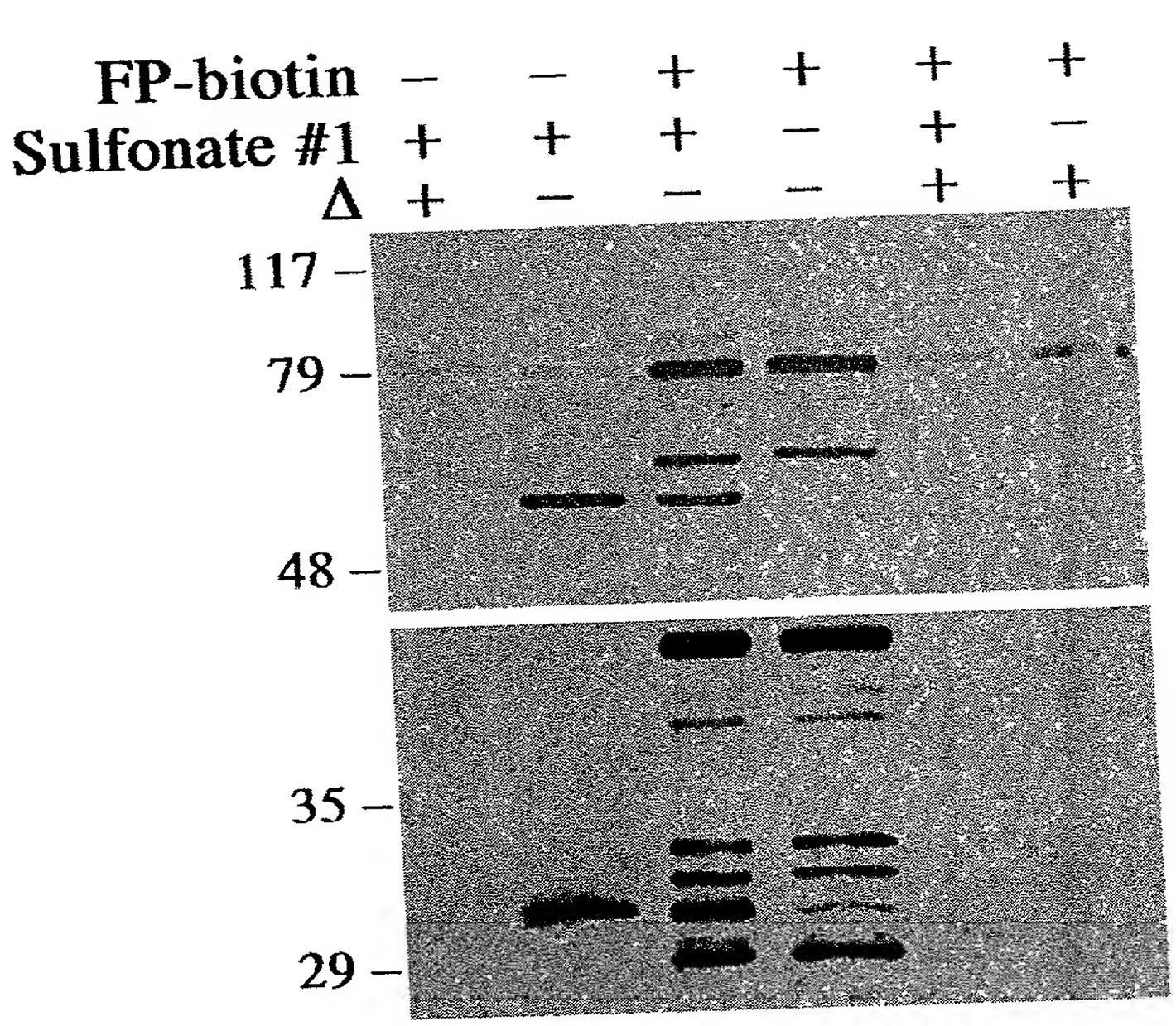


FIGURE 15

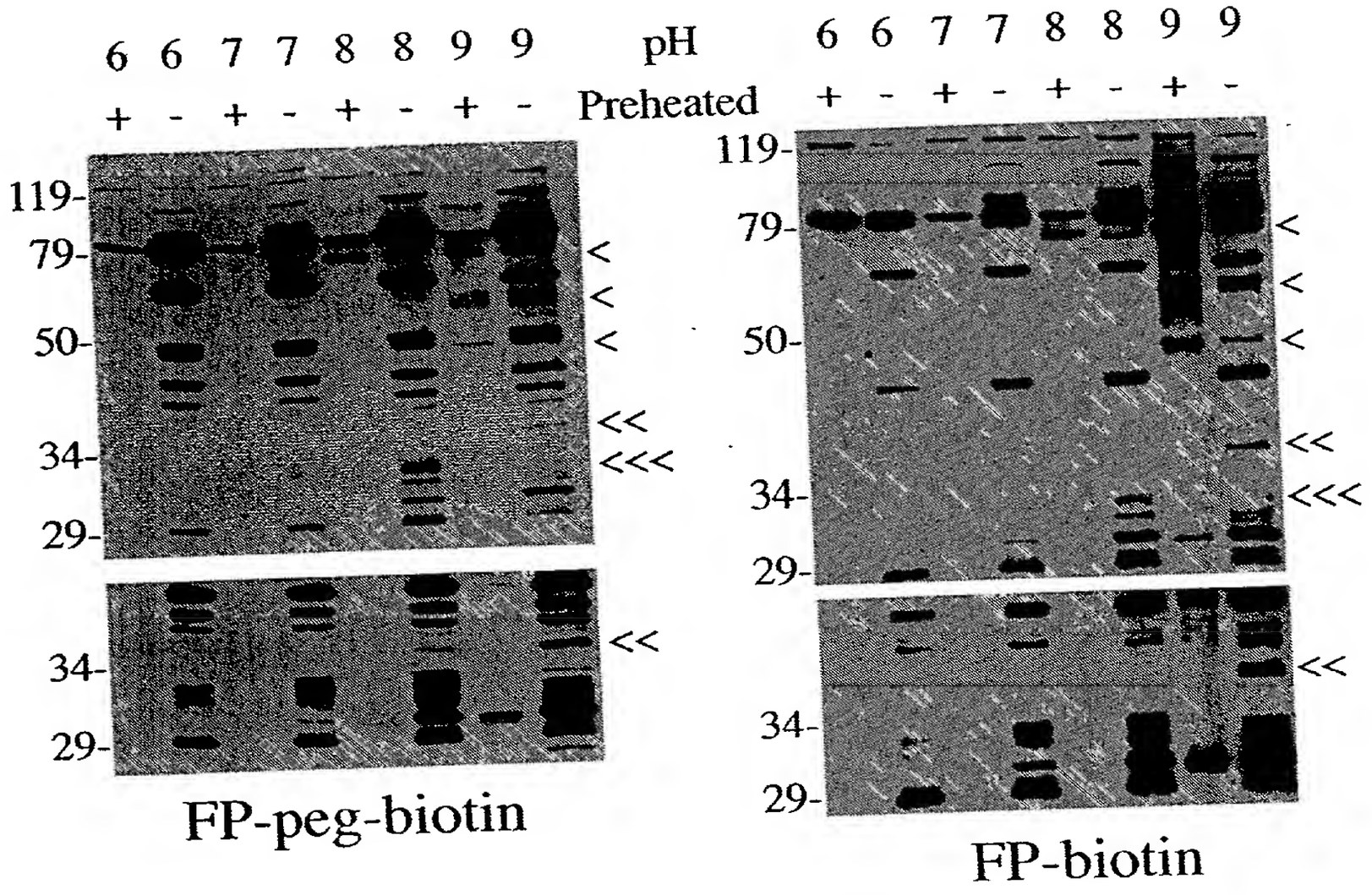

6 6 7 7 8 8 9 9
pH
+ - + - + - + - Preheated
119-
79-
50-
34-
29-
<
<
<
<<
<<<
34-
29-
<<
FP-peg-biotin
6 6 7 7 8 8 9 9
+ - + - + - + -
119-
79-
50-
34-
29-
<
<
<
<<
<<<
34-
29-
<<
FP-biotin

FIGURE 16

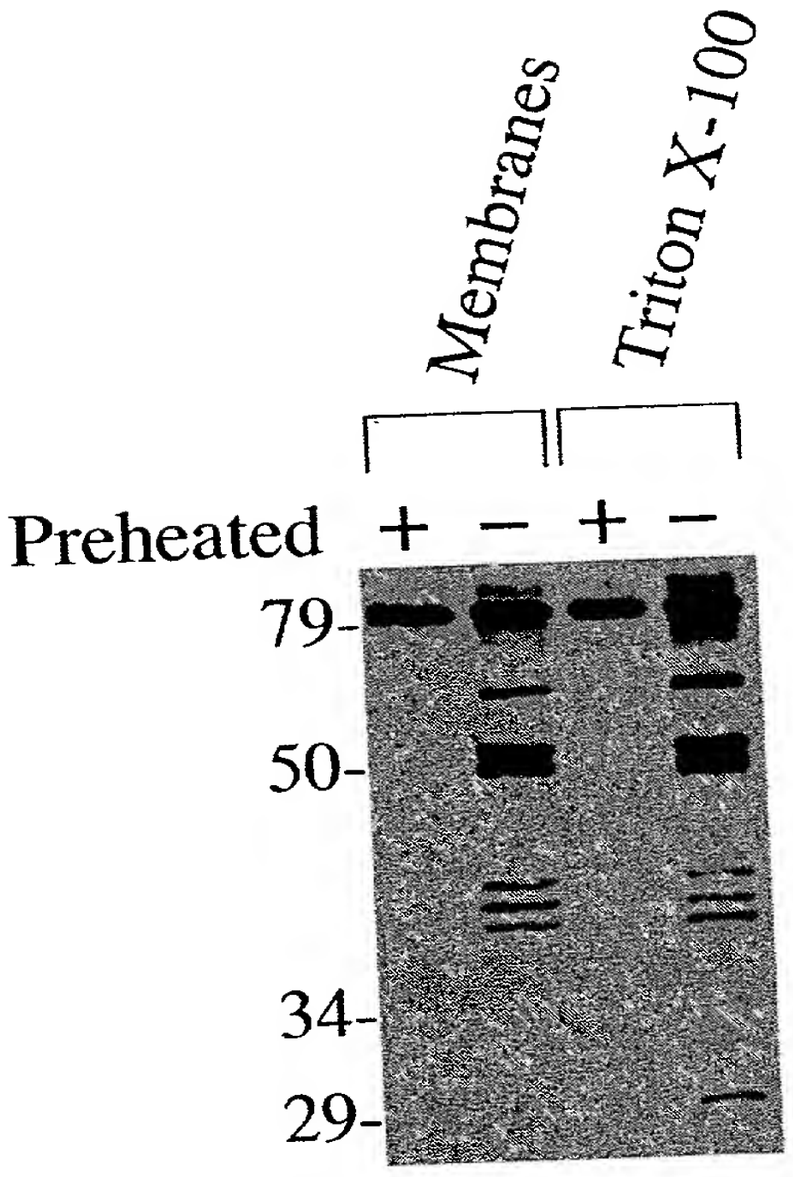


FIGURE 17

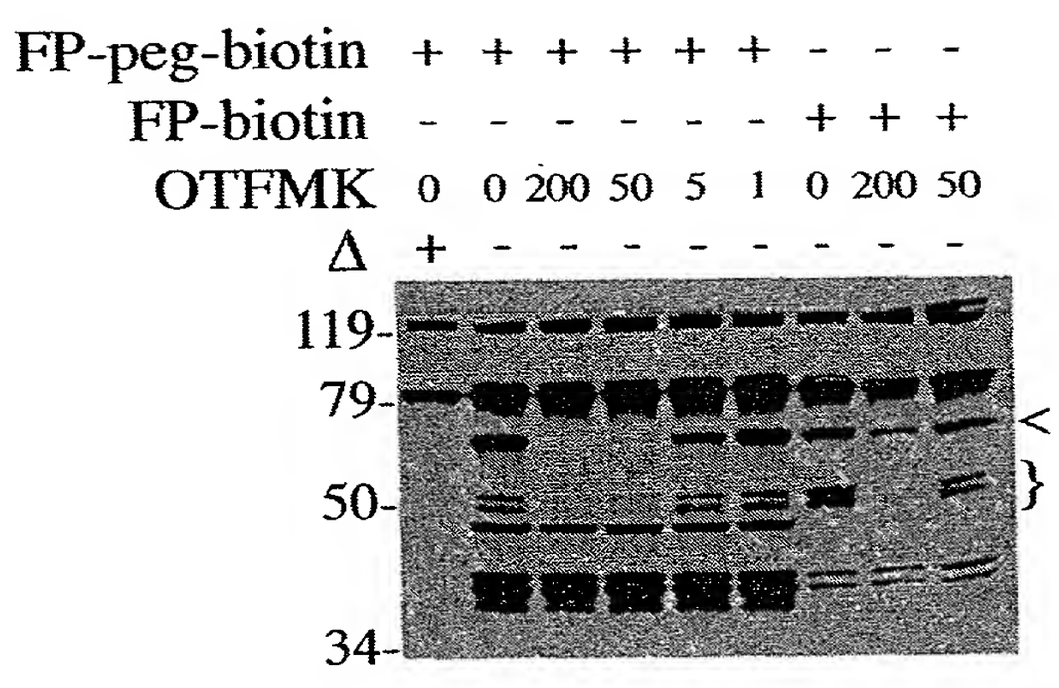


FIGURE 18

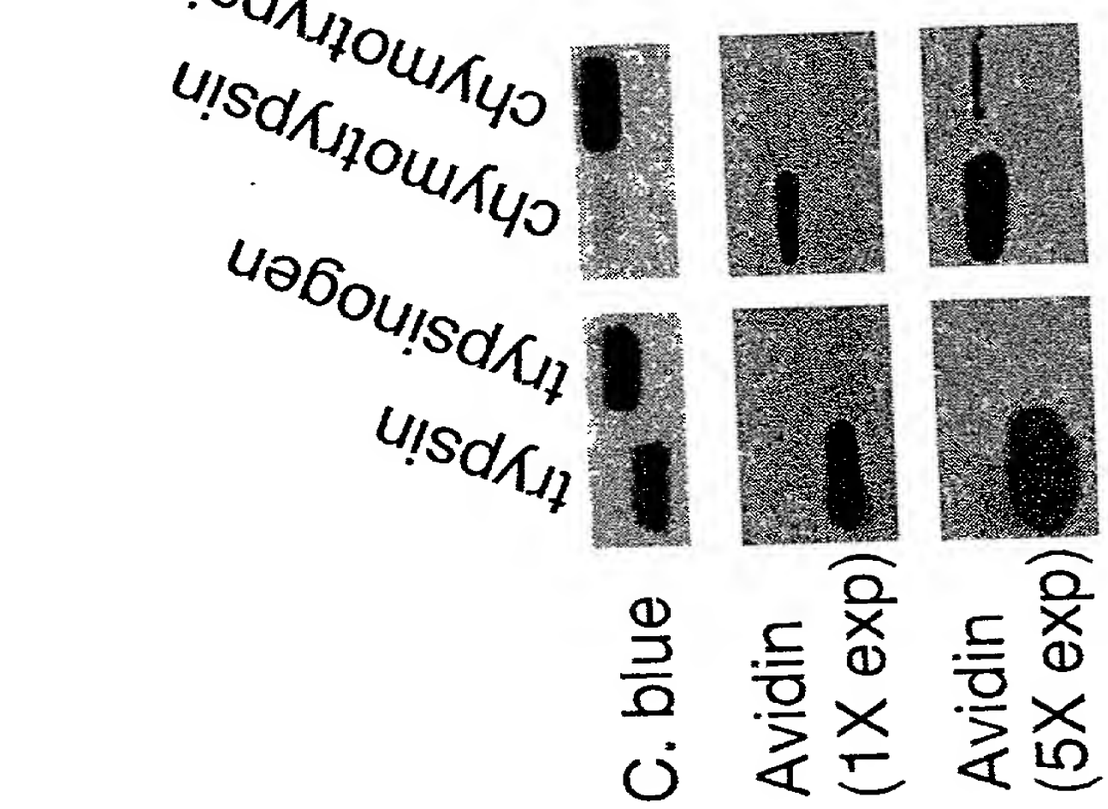
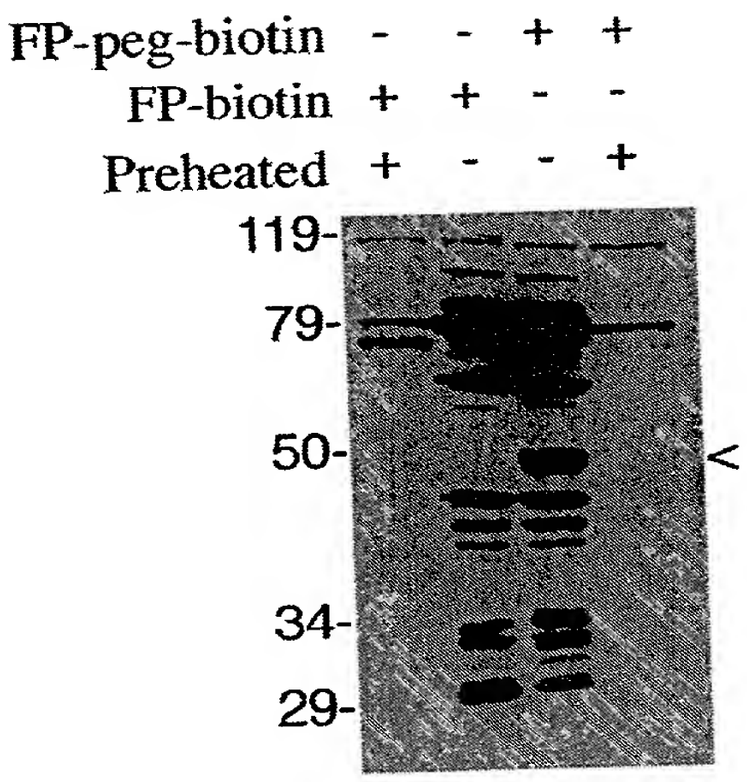
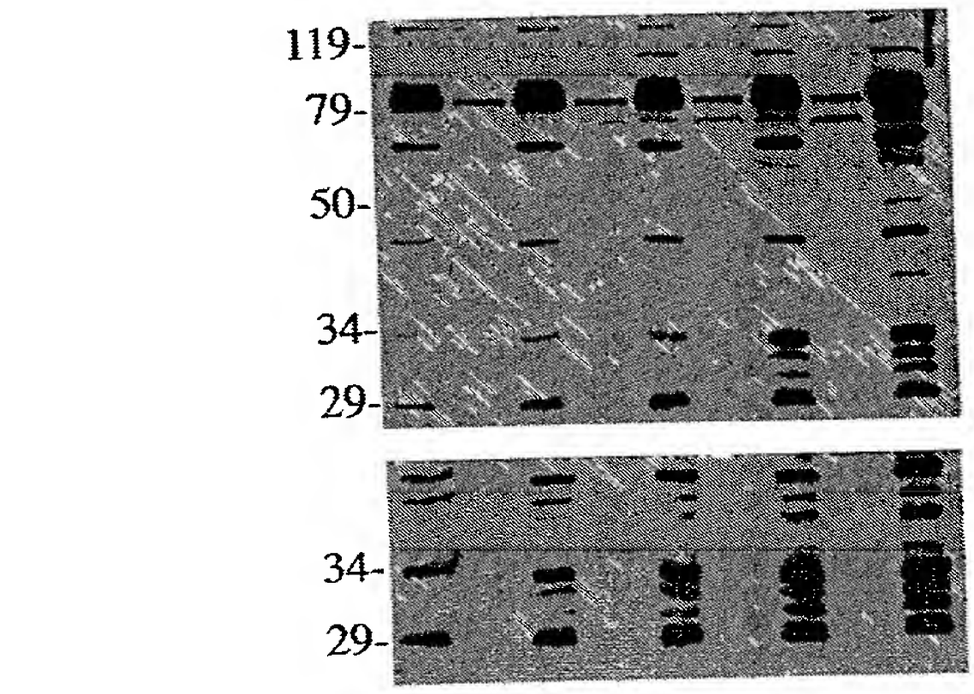

C. blue
Avidin (1X exp)
Avidin (5X exp)
trypsin
trypsinogen
chymotrypsin
chymotrypsinogen

FIGURE 19

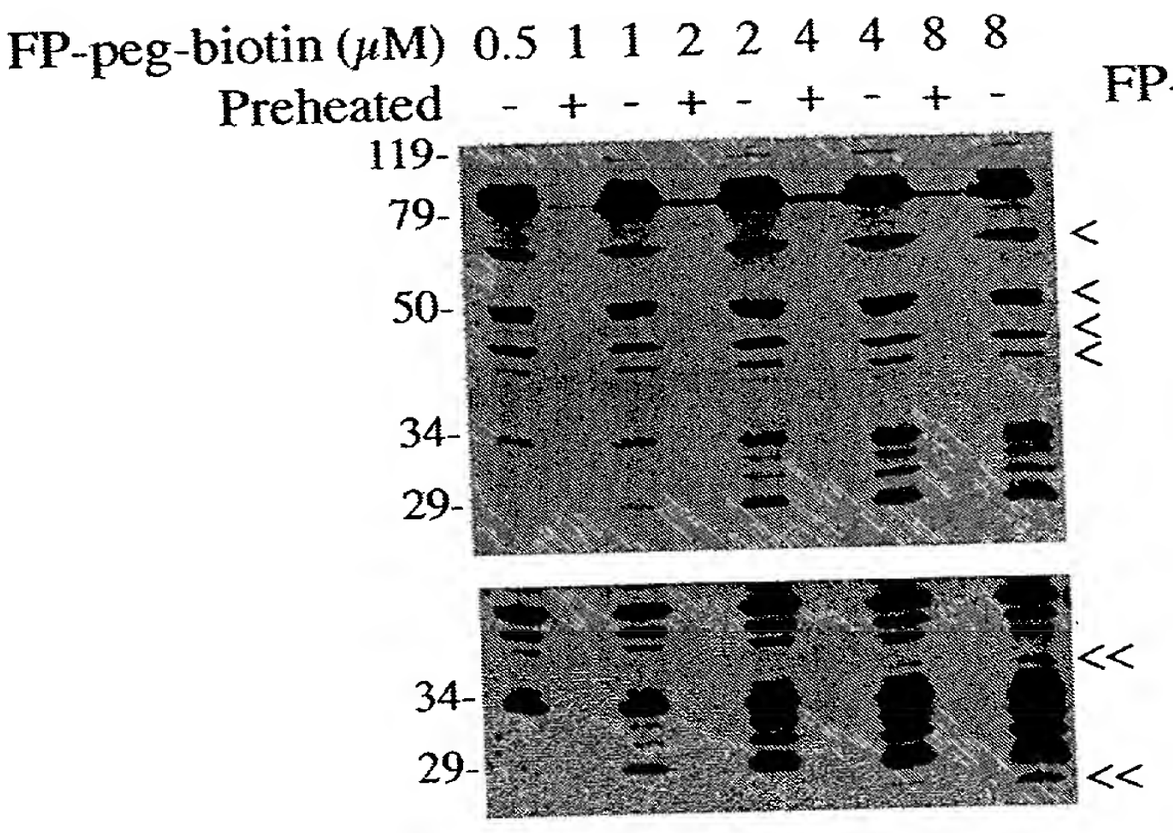
A.



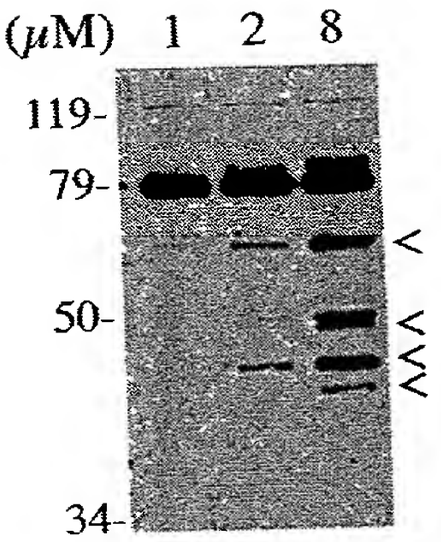
B.



C.



D.



One minute reaction

FIGURE 20

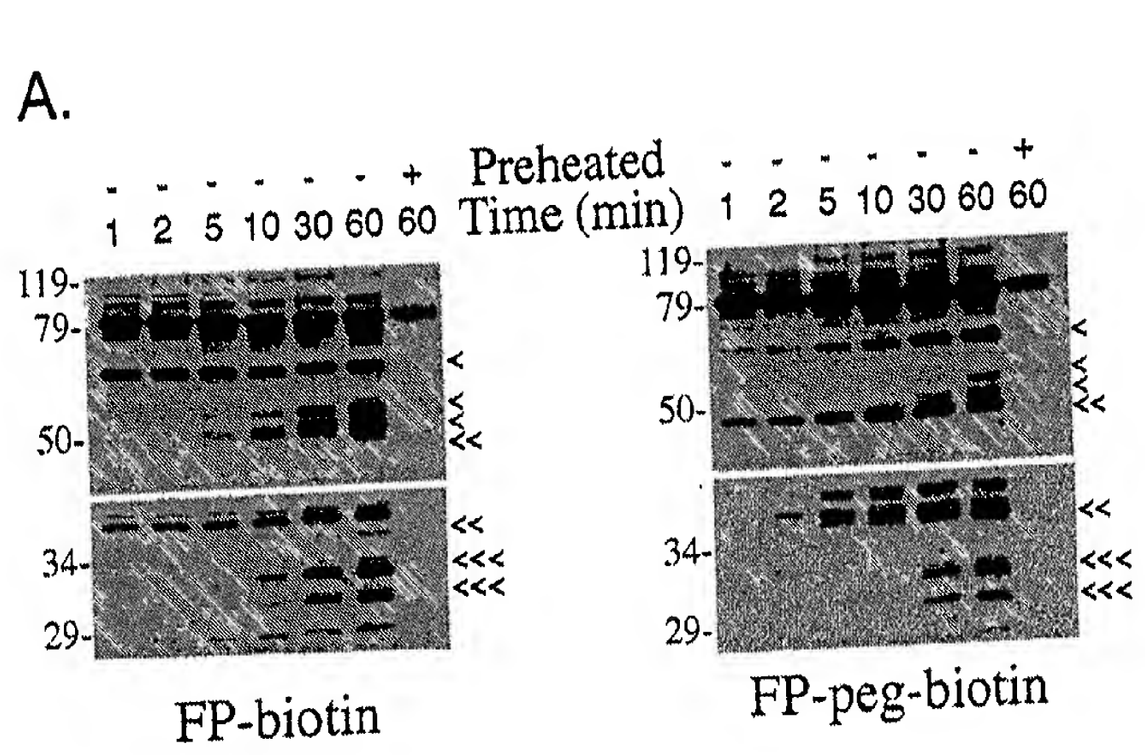

A.
Preheated - - - - - - +
Time (min) 1 2 5 10 30 60 60
119-
79-
50-
34-
29-
FP-biotin
Preheated - - - - - - +
Time (min) 1 2 5 10 30 60 60
119-
79-
50-
34-
29-
FP-peg-biotin
B.
FP-peg-biotin (μM) 4 4 0 0 2 2
FP-biotin (μM) 0 0 4 4 2 2
Preheated - + - + - +
79-
50-

FIGURE 21

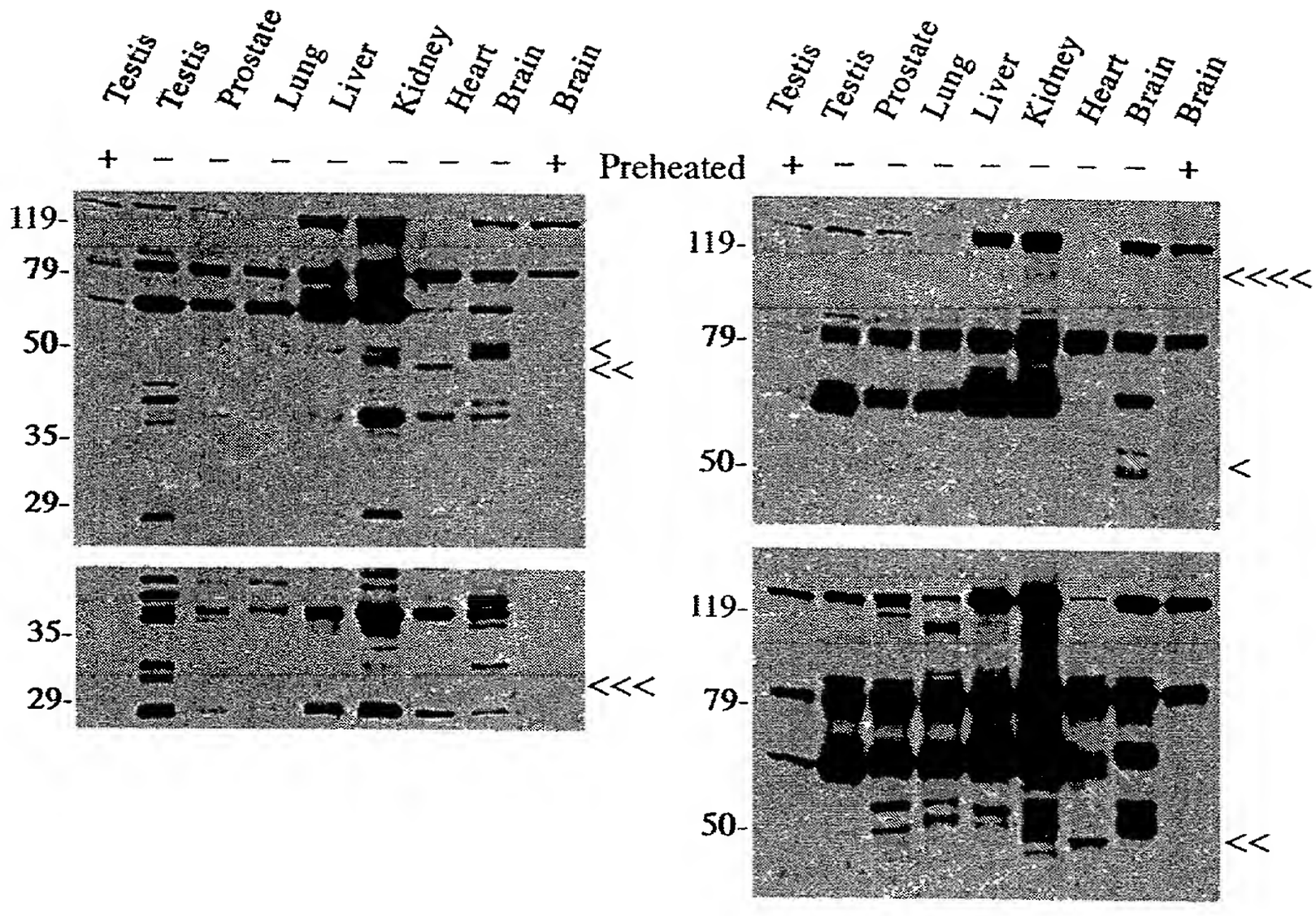
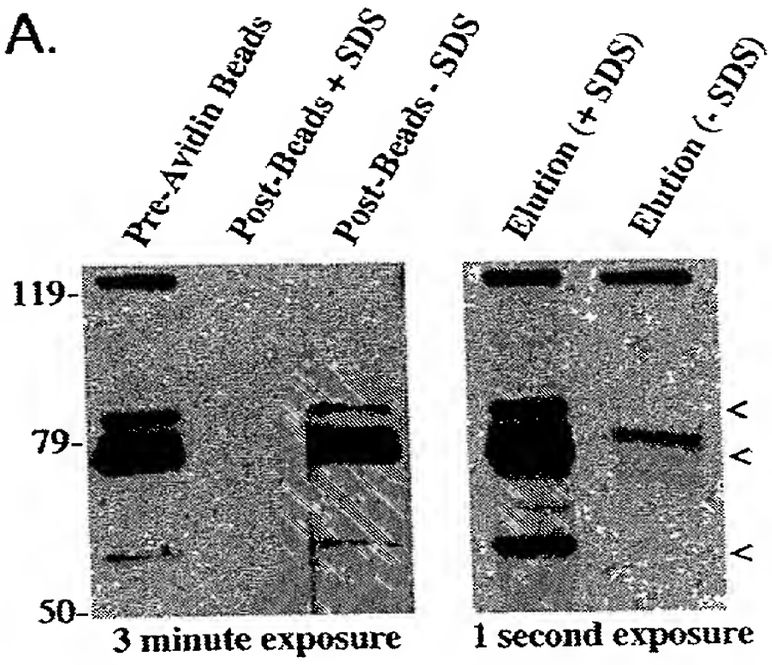

Testis
Testis
Prostate
Lung
Liver
Kidney
Heart
Brain
Brain
+ − − − − − − − + Preheated + − − − − − − − +
119-
79-
50-
35-
29-
<<<<
<<
<
<<<

FIGURE 22

A.


Pre-Avidin Beads
Post-Beads + SDS
Post-Beads - SDS
Elution (+ SDS)
Elution (- SDS)
119-
79-
50-
3 minute exposure
1 second exposure

B.

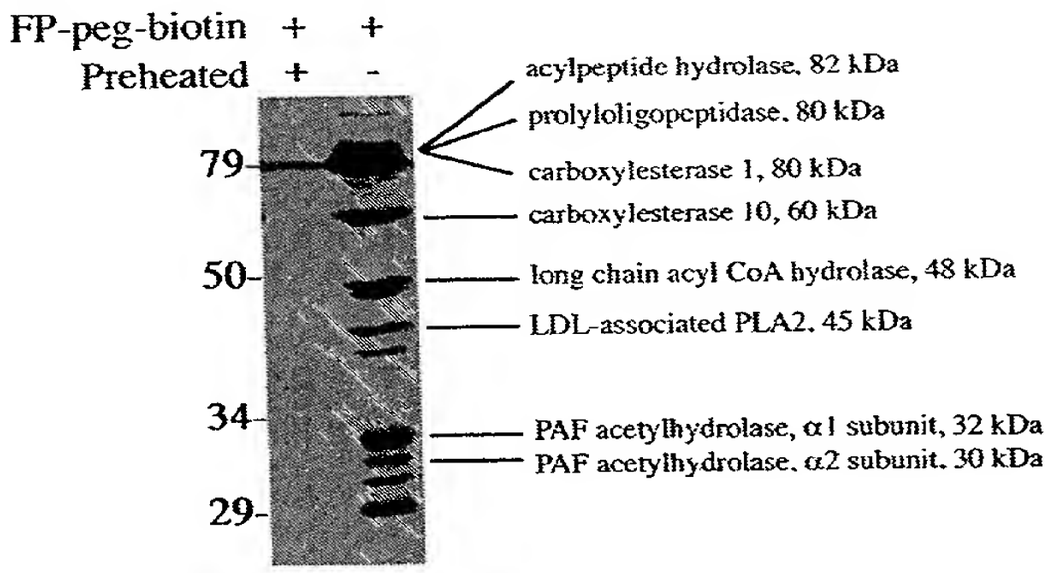

FP-peg-biotin + +
Preheated + -
79-
50-
34-
29-
acylpeptide hydrolase, 82 kDa
prolyloligopeptidase, 80 kDa
carboxylesterase 1, 80 kDa
carboxylesterase 10, 60 kDa
long chain acyl CoA hydrolase, 48 kDa
LDL-associated PLA2, 45 kDa
PAF acetylhydrolase, α1 subunit, 32 kDa
PAF acetylhydrolase, α2 subunit, 30 kDa

FIGURE 23